## SEX IDENTIFICATION IN SOME LARVAE OF SCARABAEOIDEA.<sup>1</sup>

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Recent investigations have indicated the need for sex determination of immature insects. Toxicologists, for instance, find significant differences in the susceptibility of sexes to insecticides, and physiologists require a knowledge of the sex of immature insects for hormone studies. Entomologists in the past have sexed larvae indirectly according to secondary sex characteristics, such as the development of the external genital armature, and the appearance of the antennae. Little attention has been given to the external manifestation of internally developing reproductive structures. This paper deals with the latter and employs the method of sexing suggested by Hurpin (1953).

Herold (1815), in working on the internal reproductive organs of *Pieris brassicae*, noted in the larva two long threads extending posteriorly from kidney-shaped bodies to the ninth abdominal segment where they fastened to small, white swellings under the rectum. From Herold's description and drawings one may see that he described the testis, vasa deferentia and terminal ampullae. Hurpin, in a paper on the recognition of sex in larval Scarabaeidae described the terminal ampullae as being the "Organ of Herold," but Hurpin does not explain the anatomical significance of the ampullae.

In female embryos Johannsen & Butt (1941) say that ventral remnants from the coelonic sacs of the seventh abdominal somite form the ampullae. Snodgrass (1935) states that with the posterior extension of these ampullae and their ducts to the posterior ventral part of the eighth abdominal segment, the definitive opening of the median oviduct, is shifted from its primitive opening on the venter of the seventh abdominal segment to a location on the venter of the eighth abdominal segment.

In male embryos Johannsen & Butt say that the ampullae are formed from ventral remnants of the coelomic sacs of the tenth abdominal somite and that these ampullae are attached to the posterior ends of the vasa deferentia and persist as terminal parts of these ducts but later unite to form the anterior part of the defini-

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98

tive ejaculatory duct. Snodgrass (1941) explains that where the embryonic history of the vasa deferentia has been followed in connection with the development of the phallus the terminal ampulae of the ducts leave their primary points of attachment on the ventral ectoderm of the tenth abdominal segment, or in the appendage rudiments of this segment, and migrate mesally and anteriorly to the base of the tenth segment or into the posterior part of the ninth. Here a median ectodermal ingrowth is formed that unites with the ampulae and becomes the definitive ductus ejaculatorus.

In connection with a project on the morphology and histology of the scarabaeid larva of the European chafer, *Amphimallon majalis* (Razoumowski), it was noticed that sex could be determined in live larvae by using the method described by Hurpin. The integument is transparent in these larvae, making possible the direct observation of certain internally developing reproductive structures in males. Two ovoidal structures, the terminal ampullae (Figs. 1, 3, Ta), appear lying at an angle to each other in the ventral part of the ninth abdominal segment. These structures are embedded in a sclerotized triangular plate which is formed by an invagination from the body wall and marks the location of the definitive ejaculatory duct (Figs. 1, 3, St); the vasa deferentia (Vd) extend from the testis (T) in the sixth abdominal segment to the ampullae in the ninth segment (Fig. 1).

In order to see how early in development the ampullae of the male could be found, embryos of *Amphimallon majalis* were sectioned parasagittaly. In male embryos in which dorsal closure was complete the ampullae were found in the venter of the ninth abdominal segment and were associated with an invagination of the body wall from this segment.

In female larvae the ampullae lie in the posterior ventral part of the eighth abdominal segment (Fig. 2), but cannot be seen externally for a layer of adipose tissue lies between them and the integument (Fig. 4). From a dissection, the lateral oviducts (LO) are seen to extend from the ovaries (O) in the seventh abdominal segment to the ampullae (Ta) in the eighth segment.

Dissections on many larvae were done to determine sex by identification of the ovaries and testis alone, but the location of the terminal ampullae and the fact that these structures are visible through the integument of the male and not visible through the integument of the female definitely establishes a method of sexing these larvae without recourse to dissection.



Explanation of text figures. Abbreviations: LO—lateral oviduct, N—10th abdominal nerve, O—ovaries, R—rectum, St sclerotized triangle, T—testis, Ta—terminal ampullae, V—ventriculus, Vd—vasa deferentia. Fig. 1. Internal view of male larva showing ventral abdominal floor. Fig. 2. Internal view of female larva showing ventral abdominal floor. Fig. 3. External ventral view of male abdomen showing that ampullae are visible through integument. Fig. 4. External ventral view of female abdomen showing that terminal ampullae are not visible through integument. The following species of Scarabaeoidea were sexed by the above method: Passalidae: Popilius disjunctus; Lucanidae: Ceruchus piceus; Scarabaeidae: Xyloryctes blatchleyi, Autoserica castanea, Popillia japonica, Amphimallon majalis, Anomala orientalis, Pelidnota punctata, Osmoderma species.

Data used for this method of sexing can be summarized best by the following table:

	FEATURE	MALE	FEMALE
1.	location of am- pullae	on posterior ventral part of 9th abdomi- nal segment	on posterior ventral part of 8th abdomi- nal segment
2.	are ampullae vis- ible through in- tegument	yes	<i>no;</i> a layer of adi- pose tissue lies between ampul- lae and integu- ment
3.	opening denoted by location of ampullae	definitive ejaculatory duct	definitive median oviduct
4.	ampullae formed from	coelomic sacs of 10th abdominal segment	coelomic sacs of 7th abdominal segment

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